EXPERIMENTAL TRYPANOSOMIASIS: T. EQUIPERDUM INFECTION IN THE DOG

EDWARD B. KRUMBHAAR

From the John Herr Musser Department of Research Medicine, University of Pennsylvania

In the previous studies from this department on the relation of the spleen to blood destruction and regeneration, we have used sodium oleate, toluylendiamin and hemolytic immune serum as hemolytic agents. Such agents, even when given in repeated doses, produced only a temporary transient effect, and it seemed desirable, in connection with the problem of iron metabolism and of the elimination of urobilin, to use a hemolytic agent which will cause a more chronic anemia. To this end, we have employed Trypanosoma equiperdum, which causes the disease dourine in horses, and is transmissible to lower animals. The disease which T. equiperdum produces in dogs presents so many points of interest that it seems wise to report the general picture of this experimental disease at this time. The chemical studies will appear in a later communication.[1]

The best discussion of the general literature of trypanosomiasis is still found in the book of Laveran and Mesnil.[2] The various blood changes caused by trypanosomes are reported in the recent works of Jakimoff and Wassilewsky,[3] Lafranchi,[4] and Boycott and Price-Jones.[5]

Although many experimental studies of the changes brought about by various strains of trypanosomes are found in the literature, few if any studies have been made with T. equiperdum. In a study of nagana, or tse-tse fly disease (caused by T. brucei), Kanthack, Durham, and Blandford[6] found changes similar to those produced by us in the various animals studied. These observers found that the duration of the disease depended largely on individual susceptibility. Rats survived from 6 to 26 days, mice from 8 to 25, rabbits 13 to 58, dogs 14 to 26, and guinea-pigs from 20 to 183 days. The disease could be transmitted by inoculation, subcutaneously, intravenously, intraperitoneally, in the anterior chamber of the eye, or in skin scratches. Trypanosomes were recovered from the blood, spleen, bone marrow, lymph nodes, aqueous humor, testicular juice, and all serous and edematous fluids. The salient symptoms were wasting, loss of power, fever, edema, eye changes, anemia, and urobilinuria. As in our series, it was noticed that wounds did not heal well and frequently became septic.

Received for publication August 18, 1917.

[1] Dubin, H., and Pearce, R. M.: The Elimination of Iron and Its Storage in the Liver and Spleen in Experimental Anemia, II, Jour. Exp. Med., in press.

[2] Paris, Masson & Cie, Ed. 2, 1912.

[3] Compt. rend. Soc. de biol., 1910, 128, p. 309.

[4] Fol. Hemat., 1912, Arch. 13, p. 55.

[5] Jour. Path. and Bact., 1913, 17, p. 347.

[6] Proc. Roy. Soc., 1899, 64, p. 100.

Moore and Breinl[7] have studied the life history of T. equiperdum in rats and explain its method of fission, its metamorphoses, and its three maximum phases.

METHODS

Dogs alone were used in our experimental work. At first, dogs were injected intraperitoneally with infected rat's blood containing approximately 600,000,000 T. eqiperdum. As, however, infection did not always result, all subsequent inoculations were given intravenously and the procedure adopted of injecting 1 c.c. per kilo of body weight, of whole blood from a heavily infected dog, without further attention to the actual number of trypanosomes present.

Such inoculations were successful in all instances except two: in one of these a second attempt proved successful, in the other, no further attempt was made. A few attempts to transmit the infection to guinea-pigs and rabbits were entirely unsuccessful. Our conclusions are based on the study of 28 infected dogs.

CLINICAL COURSE OF THE DISEASE

Incubation Period.—The incubation period varied from 3 to 8 days, and directly with the size of the dose. With successive transmission through dogs, the virulence of the infection was increased, so that both the incubation period and the duration of the disease was shortened, and the maximum anemia more quickly reached.

General Symptoms.—Throughout the incubation period, up to the appearance of trypanosomes in the circulating blood, the dogs appeared normal. With the appearance and multiplication of trypanosomes in the blood, the dogs began to show a marked general weakness, with considerable loss of weight, but it was also noticed that a severe anemia and even a fatal termination might be reached without such symptoms being extreme. Lethargy and tendency to sleep were conspicuous but it was difficult to distinguish between these conditions and the associated symptoms of weakness. The effects of decubitus appeared on prominent portions of the body, and although emaciation was undoubtedly a predisposing cause, there was a marked decreased tendency toward healing both in these and in various operative wounds.

A characteristic feature of the condition, which has also been noted clinically in other forms of trypanosomiasis, was the frequent occurrence of edema. In one dog this took the form of a marked general anasarca, while in others it was limited to edema of the external genitalia and localized swellings around the tendo Achillis. Living trypanosomes were readily obtained in considerable number from the edema fluid. Yorke[8] explains that this edema as due to a dilution of the toxic blood plasma following changes in osmotic pressure rather than to the actual presence of the trypanosomes.

Another almost constant condition was the appearance of marked eye lesions —keratitis, iritis, hemorrhagic exudates in the anterior chamber—changes which have been frequently commented on by other writers on trypanosomiasis. Living trypanosomes were found in the humors of eyes presenting these lesions. The nature and pathology of these eye lesions will be considered in detail elsewhere.[9]

The urine of these dogs was concentrated, of high specific gravity and high color. Sugar was never found. Albumin was present only occasionally in

[7] Proc. Roy. Soc., 1908, 80, p. 288.
[8] Ann. Trop. Med. and Parasit., 1911, 5, p. 127.
[9] Woods, A. C., and de Schweinitz, G. E.: Arch. Ophth., 1917, 42, p. 431.

very faint traces. Casts were never found. Bile pigment (Gmelin and Rosenbach tests), however, was usually present, although no staining of the skin or mucous membrane was ever noted.

In the only two animals in which death occurred in the natural course of the infection, the mode of death was very suggestive of a cerebral lesion. A few hours before death, the lethargy increased to a condition of stupor. This was succeeded by unconsciousness, complicated by semipurposeful convulsions in which the unconscious animal, lying on its side, performed rapid running motions with all legs. Between convulsions dyspnea was marked and the pulse rapid. Although this was probably due to actual cerebral involvement, no thrombi, emboli, or other gross lesions could be discovered.

Almost as soon as trypanosomes are found in the blood signs of anemia are manifest, both in the hemoglobin and red blood cell count. The hemoglobin tends at first to fall more rapidly but the fall in red cells is mainly parallel. In a few weeks, the hemoglobin may fall to 40 or lower, the erythrocytes to less than 3,000,000 per c.mm. The course and character of the anemia in a single dog is shown in Table 1, and composite curves of changes in red cells and hemoglobin in several dogs in Charts 1 and 2.

TABLE 1

T. EQUIPERDUM INFECTION IN THE DOG

Infection Time in Days after Injection of 3 C.c. Whole Blood, about 150,000,000 T. equiperdum	Hemoglobin	Red Cells	Leukocytes	Differential Counts			
				Polynuclears	Small Lymphocytes	Large and Transitional Lymphocytes	Eosinophils
Before	105	6,580,000	12,400	46	40	5	9
2	98	7,020,000	19,600	66	20	10	4
8	...	6,600,000					
11	90	5,600,000	9,600	54	40	2	4
18	54	3,800,000	6,600	52	45	2	1
26	50	3,400,000	5,400	73	27	...	...
33	46	3,440,000	8,200	79	14	7	...
40	50	3,900,000	45,200	84	11	5	...
46	...	....	.....	..	..	...	...

* Hemolysis in various strengths of salt solution, 0.30 to 0.46%.

The leukocytes after showing a temporary leukocytosis lasting only a few days, exhibit a progressive leukopenia during the ensuing period of the disease. In a few cases in which counts were made shortly before death, a terminal leukocytosis was observed (as high as 45,000), but it was not definitely ascertained whether or not this was due to terminal secondary infection (Chart 3).

Differential counts show that the initial and terminal leukocytoses are due chiefly to increase in the polymorphonuclear cells. The eosinophils tend to disappear during the course of the infection. During the leukopenic stage, the lymphocytes show both a relative and actual increase (Chart 4).

Nucleated red cells, both normoblasts and megaloblasts, occasionally appear in the blood stream soon after anemia has developed and persist for 2 or 3 weeks. They nearly always disappear a few weeks before death, in spite of the fact that the bone marrow is always found at necropsy to be hyperplastic. Polychromatophilia (which is occasionally found in the normal dog's blood) is not marked; neither is there much anisocytosis or poikilocytosis.

The resistance of the erythrocytes (measured by hypotonic salt solution), though not showing very striking changes, tended to be decreased. This was especially true of the maximum resistance, during the earlier part of the infection (Chart 5).

Blood platelets, examined by the Wright and Kinnicutt method, were apparently decreased, but the counts of these were so variable that we have not much confidence in the results obtained (Table 1).

Estimation of the percentage of skeined or reticulated erythrocytes (by vital staining with brilliant cresyl blue) showed in the earlier stages of the infection an increase, indicating an attempt at bone marrow regeneration. This latter was seldom sufficient, however, to cause the appearance of normoblasts in the peripheral blood stream. Seidelin[10] also has found a reduction in platelets and an increased number of 'metachromatic erythrocytes' in trypanosome infection (Table 1).

Evidences of blood destruction, as measured by increased elimination of urobilin and increased storage of iron in liver and spleen were present. These results will be considered elsewhere.[1]

TABLE 1—*Continued*

T. EQUIPERDUM INFECTION IN THE DOG

Fragility		Skein Cells, per Cent.	Platelets	Weight of Dog, Kg.	Remarks
Complete Hemolysis*	Beginning Hemolysis*				
32	46	0	80 M.	9.7	
34	46	...	.....	10.2	Trypanosomes in circulating blood
36	46				
36	46	1.5	.....	....	
32	44	2	.....	11.2	Keratitis
32	44	0.6	73 M.		Diarrhea
30	44	0.4	50 M.	10.3	
	..	...	.....	...	Died

THE EFFECT OF SPLENECTOMY

In the course of the studies of the iron metabolism in these animals, a number of dogs were splenectomized, some before and some after infection with trypanosomes. In both instances the animals did poorly. Six dogs splenectomized at the height of the trypanosome infection died within 2 weeks, and the majority within 1 week. These dogs showed no appreciable change in the blood picture beyond the usual leukocytosis following splenectomy. Likewise, with one exception, the 5 dogs splenectomized before infection with trypanosomes all died within 11 days after inoculation, and within 2 or 3 days following the appearance of trypanosomes in the circulating blood. The exception was a dog splenectomized on January 22. It showed the early leukocytosis and a gradually developing, moderate anemia characteristic of splenectomized dogs. It was inoculated with trypanosomes on March 31, and lived 31 days after inoculation. During this period the anemia gradually grew more intense, the hemoglobin falling to 35% and the red blood cells to 3,940,000, on the day of death.

[10] Jour. Path. and Bact., 1914-1915, 19, p. 315.

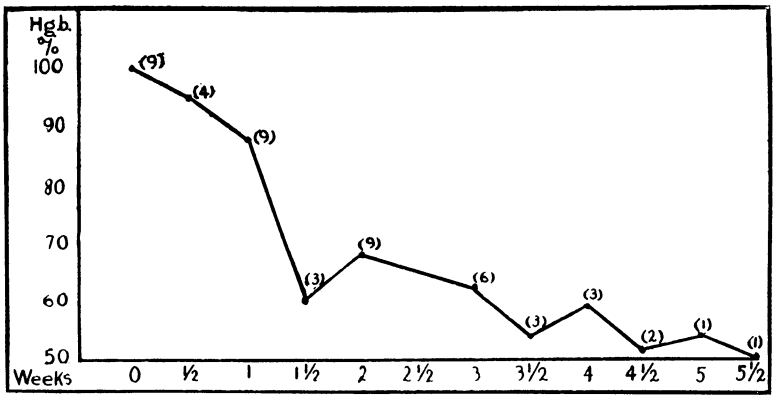

Chart 1.—Hemoglobin counts after trypanosome infection. Composite chart. Figures in this and subsequent charts indicate the numbers of animals examined.

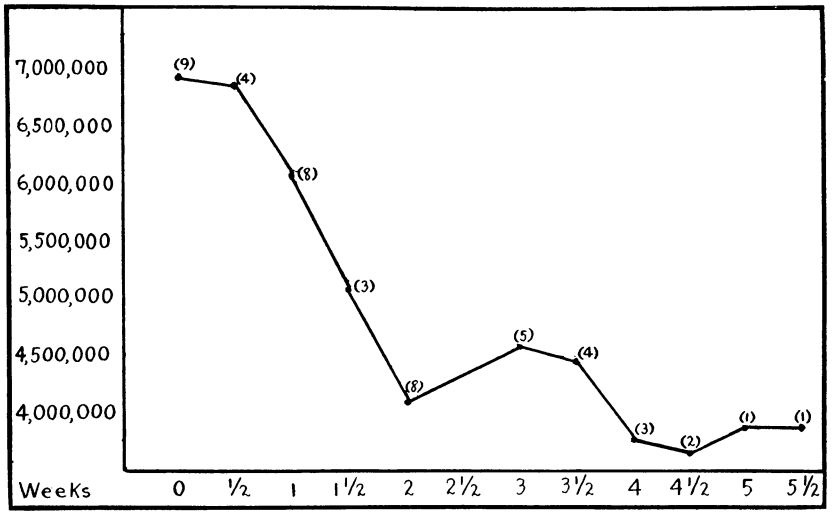

Chart 2.—Red blood counts after trypanosome infection. Composite chart.

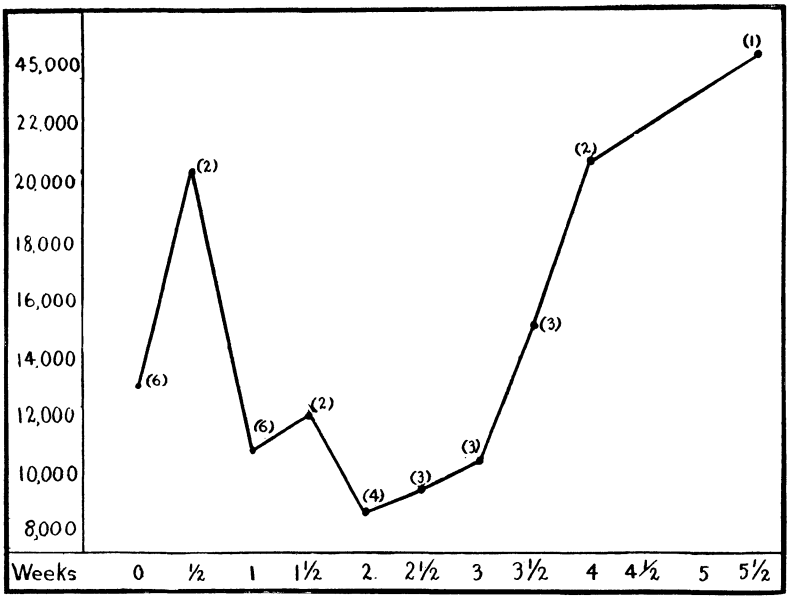

Chart 3.—Leukocyte counts after trypanosome infection. Composite chart.

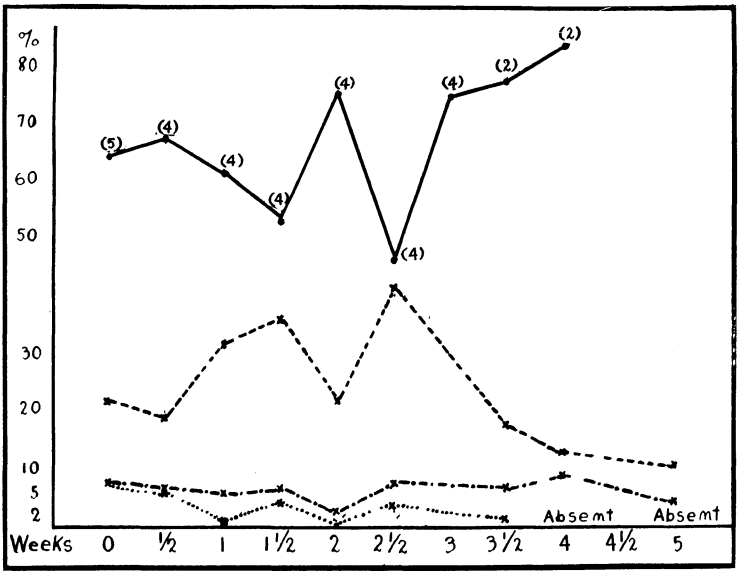

Chart 4.—Differential counts after trypanosome infection. Composite chart. Polymorphonuclear leukocytes expressed with straight line ————; small lymphocytes expressed with dashed line — — — —; large and transitional forms expressed with broken line — . — . — . — . ; eosinophils expressed with dotted line .....

THE EFFECT OF SALVARSAN ON THE DISEASE

Riquer[11] in Germany, and Schamberg, Kolmer, and Raiziss,[12] in this country, have shown that salvarsan, and its American counterpart, arsenobenzol, have a chemotherapeutic effect on trypanosomiasis. Given intravenously in the dosage of 0.01 gm. per kilo of bodyweight, the blood of dogs infected with T. equiperdum becomes quickly sterile often after one injection. To insure a permanent cure, we have found it necessary to give 3 injections on successive days, and to follow this with 3 or more injections at subsequent 3-day intervals.

Following the intravenous administration of arsenobenzol, all clinical manifestations and symptoms disappeared in a most prompt and striking manner. Within 3 days after the first injection the lethargy and weakness disappeared, the edema subsided, the decubitus lesions began to heal rapidly, and the ocular symptoms cleared up. After 10 days of treatment the dogs appeared normal.

The blood pictures in the dogs, in which a permanent cure was effected, have not been followed. Table 2 shows the prompt regeneration in the blood following 3 injections of arsenobenzol. The trypanosome infection later recurred in this dog with the development of a progressive anemia.

TABLE 2

REGENERATION OF THE BLOOD AFTER ARSENOBENZOL

	Hb %	Red Cells	Leukocytes
February 28. Before treatment.....	32	2,700,000	4,500
0.01 gm. arsenobenzol per kilo of body weight on March 1, 2 and 3			
March 3	63	4,290,000	19,000
March 10	58	5,310,000	20,300
March 17	78	5,270,000	12,600

PATHOLOGIC ANATOMY

On postmortem examination the most important changes found were: (1) degenerative changes in the parenchymatous organs; (2) hyperplasia of the marrow and the long bones; and (3) enlargement of the spleen. The 1st and 2nd of these do not demand detailed discussion, as they are the usual concomitants of experimental anemia. The splenomegaly, however, is worthy of special comment. In the normal dog the weight of the spleen varies greatly with an average of about 25 gm. In a special series we have found the extremes to be 11 and 55 gm. respectively, the majority, however, falling between 17 and 26. The size of the normal spleen varies likewise; its length being 11-19 cm., its greatest widths 3-6 cm., and thickness 0.6-1.8 cm. In animals infected with trypanosomes we have found the weight to be greatly increased. In 8 animals it ranged from 110 to 150 gm. and in 5 from 50 to 87 gm. The increase in size was not in proportion to the weight, the largest spleen (150 gm in weight) measuring 26 cm. in length, 7.5 cm. in greatest width and 4.5 cm. in thickness. In its gross appearance the spleen is purplish-red, with a smooth distended capsule and on section soft, succulent, and of bright red color, with very prominent malpighian bodies. Microscopically, congestion is the chief characteristic but there is also an increase of endothelial cells and of old blood pigment. Many of the endothelial cells are phagocytic for erythrocytes and the malpighian bodies appear unusually cellular.

[11] Ztschr. f. Immunitätsforsch. u. exper. Therap., 1913, O., 21, p. 92.

[12] Jour. Am. Med. Assn., 1915, 65, p. 2142.

SUMMARY

Dogs may be readily infected with T. equiperdum and a severe anemia be produced. The incubation period varies from 3 to 8 days, and a fatal termination results in from 3 to 7 weeks. By successive transmission through dogs the virulence of the infection may be increased so that both incubation period and duration of the disease may be shortened and the maximum anemia more quickly reached.

With the appearance of trypanosomes in the circulating blood, the animals show general weakness, loss of weight, lethargy, and a lessened

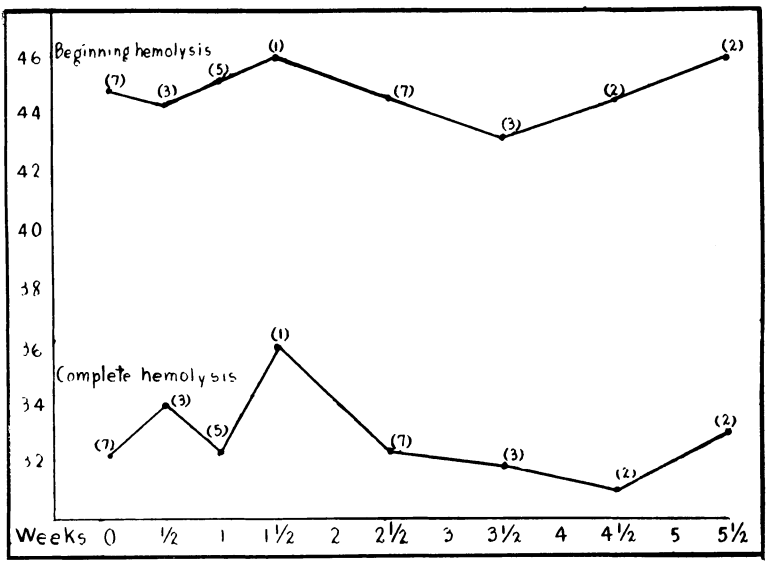

Chart 5.—Resistance tests after trypanosome infection. Composite chart. Of two animals examined 5½ weeks after infection, one showed a slight increase of both maximal and minimal resistances, the other a decrease of both resistances, so that it seems better not to include these figures.

tendency to the healing of wounds. Subcutaneous edema is a common manifestation and may appear as a general anasarca or be limited to the genitalia or the extremities. The edema fluid contains living trypanosomes. Another interesting and almost constant lesion is keratitis. Choluria is constantly present without evidence of jaundice in the skin or mucous membrane.

The anemia which develops is progressive and of the hemolytic type. The hemoglobin may fall to 40, and the red cells to less than 3,000,000 per c.c.

Attempts at regeneration are shown by the increased number of skeined cells, occasional nucleated reds in the peripheral stream, and hyperplastic bone marrow at necropsy. That this attempt at repair is entirely inadequate, however, is shown not only by the progressive lowering of hemoglobin and erythrocyte count, but also by the leukopenia, diminution in platelets, later lowering of percentage of skeined cells, and disappearance of nucleated red blood cells from the peripheral blood stream.

The principal pathologic changes are the usual degenerative lesions of anemia in the parenchymatous organs, hyperplasia of the bone marrow and a great enlargement of the spleen.

Splenectomy at the height of the trypanosome infection has no beneficial influence, but rather the reverse, on the anemia or the course of the infection. Animals splenectomized before infection died more quickly than did those with intact spleen; as a rule, within 2 or 3 days after the appearance of trypanosomes in the circulating blood.

The intravenous injection of arsenobenzol, 3 injections at intervals of 3 days, has been followed by a disappearance of all symptoms and of trypanosomes from the blood and of a prompt improvement in the blood picture. In some instances, however, the disease has recurred on the discontinuance of treatment.